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A. NITROSAMINES

The current trapping procedure for tobacco specific nitrosamines, 250 ml buffer in first trap, 100 ml buffer in second trap, and a TPM pad backup is being investigated.[1] The model cigarettes 2R1 and a nitrate added bright X6D3NX were weight and RTD selected for this study (2R1 1.173 g ± 1 mg 3.4" H_2O ± 0.1"; X6D3NX 1.139 g ± 3 mg 3.1" H_2O ± 0.1"). These cigarettes are being used for seven replicate smokings, processing, and will then be analyzed (gc-tea). This will provide a data base on trapping patterns and method reproducibility. The X6D3NX samples are being smoked with and without filters to also evaluate this important parameter.

A reference sample of N-Nitrosoanatabine (NAtB) was examined by probe eims.[2] The mass spectrum was in agreement with the literature for NAtB although a minor impurity was present. A glass column packed with 3% SP-2250, as used in our gc-tea analysis, was used for gc/eims of NAtB. A single component eluted, but the spectra obtained were no longer consistent with its identification as NAtB. The molecular ion now appeared at m/z 156 and the ms is consistent with identification as bipyridyl. While we cannot yet explain this conversion, it may relate to the low signal intensity observed for NAtB in the gc-tea system.

B. CHROMATOGRAPHY

The current procedure for quinoline was compared to chromatography of a base fraction on alumina. It appears that quinoline will elute with ether and harman with methanol. This procedure may be more straightforward than the present method.[3]

A sample of the methanol eluate (alumina column) from X6D3IM bases was used for capillary gc (HP 25 m x 0.2 mm SP-2100).[4] Approximately 200 peaks were detected, but peak intensity indicates that a considerable portion of the injected material did not chromatograph. Harman and norharman were identified by retention time as major fraction components. A synthetic sample of 2-amino-α-carboline eluted as a symmetrical peak under these conditions but no significant peak with the appropriate retention time was observed in the methanol eluate fraction.

This methanol eluate was also analyzed by gc/eims (glass column packed with 3% SP-2250 DB).[2] The chromatogram contained several well defined peaks. Harman and norharman were again observed as major chromatographable components. Further analysis of ms of fraction components and comparison of gc results to capillary gc results is planned.

Considerable time was devoted to the development of new HPLC methods for separation of individual components in complex base fractions, again the methanol eluate from alumina chromatography of X6D3IM base fraction was used.[5] Ion pair systems were evaluated (sodium hexanesulfonate) but the base fraction components such as 2-amino-α-carboline appear to be too hydrophobic for this approach. Next, a number of ion suppression systems were evaluated. Using ammonium carbonate for ion suppression, a three component organic solvent mixture, gradient conditions, and elevated temperature (60°) allowed optimal resolution of any selected component in this complex mixture.

C. FLUORESCENCE DETECTOR EVALUATION[3,5]

A Perkin Elmer 650-10S fluorescence spectrophotometer was evaluated this month. Good excitation and emission spectra were obtained using the standard cell for PAHs, heteroaromatics, and 2-amino-α-carboline. The instrument was used with the HPLC flow cell in an attempt to locate 2-amino-α-carboline in the methanol eluate (alumina) of the X6D3IM base fraction. Using optimal excitation (325 nm) and emission (367 nm) the peak for the 2-amino-α-carboline was easily located in this fraction at the appropriate retention time. No significant peak could be observed using the variable wavelength UV detector with this same fraction.

Comparison of the results from capillary gc, packed column gc/eims, HPLC with variable wavelength UV, and HPLC with fluorescence detection provided strong support for the analytical capability of fluorescence detection for trace components in the base fractions.

D. BASE FRACTION AND THE *SALMONELLA* ASSAY (with 6906)

A study of WSC from LTF-IIA + proline (X6D9FDB) was initiated.[6] This is a major amino acid of bright filler and was known to produce a high activity condensate when added to an LTF-IIA matrix.[7] The WSC was fractionated to acid-neutral and base fractions. As expected, the base fraction had high activity (42 rev/µg) and a high accountability (71% of WSC activity). The bases were further separated by chromatography on ODS-silica and these fractions are being tested.

Further preparative fractionation of various active "strippings" fractions from LH-20 fractions continues.[6] Samples are being submitted both for activity testing and gc/eims profiling. A number of components are being observed in these fractions by gc/eims.[2] For example, a MW 197 component (possibly a methyl amino carboline), MW 183, MW 195, and MW 209 components were located. Additional ms interpretation is in progress.

E. PRECURSORS OF WSC ACTIVITY

Equipment was obtained and set up for preparation of LTF sheet materials.[5] With assistance from Mr. G. Keritsis and Mr. J. Leik, a high organic formulation was prepared, cast, and cut.[8] This nitrogen-free filler has been submitted for analysis, cigarettes have been prepared and smoked, and the IT WSC is being tested. This material incorporates a high level of microcrystalline cellulose (Avicel-PH-101, 50 micron, FMC) instead of α-cellulose.[9] This material is being prepared in support of several ongoing precursor studies in Projects 6908 and 6906.

F. REFERENCES

1. Baker, P. G. Notebook No. 7545, p. 6.
2. McKay, C. Notebook No. 7477, p. 59.
   Kinser, R. Notebook No. 7476, p. 48.
3. Millham, J. Notebook No. 7139, p. 183.
4. Levins, R. Notebook No. 7351, p. 121.
5. Warfield, A. Notebook No. 7434, pp. 94-96.
6. Tafur, S. Notebook No. 7423, pp. 170, 171.
7. Ferguson, R. Notebook No. 7372, pp. 33, 34.
8. Petri, D. Notebook No. 7331, p. 156.
9. Gager, R. Notebook No. 7375, pp. 83, 84.

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